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miR-424 protects PC-12 cells from OGD-induced injury by negatively regulating MKP-1

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Abstract. – OBJECTIVE: It's of great significance to investigate the novel targets of drugs for the treatment of stroke. In this study, we explored the neuroprotective role of miR-424 in oxygen glucose deprivation (OGD)-induced injuries in PC-12 cells.

MATERIALS AND METHODS: PC-12 cells were subjected to OGD stimulation to mimic ischemic injury. The expressions of miR-424 and mitogen-activated protein kinase phosphatase-1 (MKP-1) were altered by transient transfection with miR-424 mimic, miR-424 inhibitor, pEX-MKP-1, or sh-MKP-1. Cell counting kit-8 (CCK-8) assay, flow cytometry, and quantitative reverse transcription polymerase chain reaction (qRT-PCR), were conducted to respectively detect cell viability, apoptotic cells, and the expression of miR-424 and MKP-1. The protein expressions of several factors were determined by Western blot. Meanwhile, relative luciferase activity assay was done to verify the predicted targets association.

RESULTS: OGD induced injury in PC-12 cells by suppressing cell viability and inducing apoptosis. OGD also induced the expression of miR-424 in PC-12 cells. Overexpression of miR-424 protected PC-12 cells from OGD-induced injury by increasing cell viability and decreasing apoptosis. MKP-1 was a direct target of miR-424, and its expression was negatively regulated by miR-424. Up-regulation of expression of MKP-1 aggravated OGD-induced cell injury by inhibiting the expression of hypoxia-inducible factor 1α (HIF-1α), thus inhibiting the PI3K/AKT/mTOR pathways.

CONCLUSIONS: miR-424 protected PC-12 cells from OGD-induced injury through direct suppression of MKP-1 expression, as MKP-1 promoted OGD-induced cell injury by inhibiting the expression of HIF-1α and PI3K/AKT/mTOR pathways.

Key Words:
Stroke, OGD, miR-424, MKP-1, HIF-1α, PI3K/AKT/mTOR pathways.

Introduction

Stroke is the second leading cause of death across the world in people above 60 years and fifth leading cause of death in people aged between 15 and 59 years old[1-3]. It is also considered to be one of the most important causes of acquired disability among adult population[1]. Ischemia is the most common cause of stroke; about 80% of the strokes are diagnosed to be ischemic stroke in nature[4]. Ischemic strokes mainly arise from the occlusion of the major branches of cerebral artery. Cerebral artery occlusion induced the blood supply obstacle would further results in ischemia-hypoxia injuries of brain. A series of complex cellular and biochemical molecular mechanisms are involved in the oxygen deprivation induced cell injuries, such as formation of reactive oxygen species, production of glutamate, intracellular accumulation of calcium, and triggering of inflammatory reactions[5]. All these pathophysiological changes lead to irreversible damage (infarction) to the brain tissue and cause cell death and loss of neurological functions. Common presenting features of strokes include sensory and motor loss, facial paralysis, ataxia, impaired speech, and visual impairment. What's more, prolonged hypoxia leads to long-term unconsciousness, seizure, coma, and eventually resulting in brain death[6]. The zone of ischemic penumbra (consisting of the infarcted area and the surrounding ischemic brain tissue) can be salvaged with appropriate treatment strategy within a specific time frame[7]. Treatment approaches of ischemic stroke include neuroprotection and reperfusion of the ischemic/infarcted brain tissue. Drugs and mechanical devices are used for reopening of the occluded blood vessels[7,8]. Recombinant tissue plasminogen activator (rtPA) is approved for dissolution of the occluding

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thrombus[8]. However, the time-window of rtPA is within 4-5 hours, making administration of rtPA feasible only in 5% of the total stroke patients[9]. Hence, it is urgently needed to develop the optimum treatments for ischemic stroke.

Micro RNAs (miRNAs or miRs) consist of 17-23 nucleotides which are responsible for post-translational regulation of mRNA expressions[10]. MiRNAs modulate mRNAs expression through direct binding with the 3'UTR region of the specific mRNAs[10]. Several studies have reported that the altered expression of different miRNAs in cerebral ischemia leads to either neuroprotection or neuronal damage in post-ischemic periods[11-13]. miR-424 has been indicated as a tumor marker based on its involvement in cell proliferation, differentiation, migration, and in regulation of cell cycle[14]. MiR-424 expression was down regulated both in brain tissue and in circulation in clinical samples or animal models of ischemic stroke[14,15]. miR-424 has been found to protect neuronal tissue in cerebral ischemia by suppressing microglial invasion and also by protecting from oxidative stress-induced injury[14,15].

Oxygen-glucose deprivation (OGD)-induced cerebral injury is a frequently model used in the research on ischemic stroke *in vitro*[16-19]. In the OGD model, suppression of oxygen and glucose delivery to the brain tissue leads to neuronal damage similar to ischemic stroke[16-20]. In the present work, we explored the effect and mechanism of miR-424 in OGD-induced cell damage of PC-12 cells.

Materials and Methods

Cell Culture and OGD Treatment

PC-12 cells, which are derived from a pheochromocytoma of the rat adrenal medulla, were purchased from Kunming Institute of Zoology (Kunming, China). The cells were seeded onto flasks at a density of 1×10^4 cells/mL in Dulbecco's modified Eagle's medium (DMEM; HyClone, South Logan, UT, USA), supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Gaithersburg, MD, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technologies Corporation, Carlsbad, CA, USA). They were incubated at 37°C temperature in a humidified incubator containing 95% air and 5% CO_2. Culture medium was replaced by a glucose-free DMEM with free FBS before OGD. Then, cultured cells were incubated in a hypoxia incubator which contained a mixture of 5% CO_2 and 95% N_2 (v/v) at 37°C to stimulate hypoxia injury. The culture medium was refreshed with normal medium before completing OGD and then the cells were returned into the incubator with 95% air and 5% CO_2. The normal medium under normoxia served as control.

Cell Counting kit-8 (CCK-8) Assay

Cells were seeded in a 96-well plate at a density of 5000 cells/well; cell viability was assessed by CCK-8 assay (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Briefly, after stimulation, the cells in 96-well plates were added with 10 μl of CCK-8 solution in the culture medium, and were incubated for another 1 h at 37°C in a humidified environment containing 95% air and 5% CO_2. Absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

Apoptosis Assay

Identification and quantification of apoptotic cells was detected by flow cytometry analysis using Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). In brief, the cells were seeded in a 6-well-plate at a density of 100,000 cell/well. Then, the treated cells were washed with cold phosphate-buffered saline (PBS) for twice and resuspended in 1 x binding buffer. Then, 5 μl Annexin V and 5 μl of PI were added to the resuspended cells and incubated for 30 min at room temperature in the dark according to the manufacturer's instructions. The apoptotic cells and necrotic cells were differentiated by flow cytometer (Beckman Coulter, Brea, CA, USA). The data were analyzed by using FlowJo software (Tree Star Inc., Ashland, OR, USA).

MiRNAs Transfection

miR-424 mimic, miR-424 inhibitor, and the corresponding negative controls (Scramble for miR-424 mimic, and NC for miR-424 inhibitor) were synthesized by GenePharma Co. (Shanghai, China) and were transfected into PC12 cells. Cell transfections were conducted using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Transfection and Generation of Stably Transfected Cell Lines

Full-length mitogen activated protein kinases (MAPK) phosphatase-1 (MKP-1) sequences and short-hairpin RNA directed against MKP-1 were

ligated into the pEX-2 and U6/GFP/Neo plasmids (GenePharma), respectively. The recombinant plasmids were referred to as pEX-MKP-1 and sh-MKP-1, respectively. Empty pEX-2 plasmid was acted as a negative control of pEX-MKP-1, which was referred to as pEX. Meanwhile, the U6/GFP/Neo plasmid carrying a non-targeting sequence, which referred as shNC, acted as a negative control of sh-MKP-1. Lipofectamine 3000 reagent (Life Technologies, Carlsbad, CA, USA) was used for cell transfection following the manufacturer's instructions. The stably transfected cells were selected by the culture medium containing 0.5 mg/mL G418 (Sigma-Aldrich, St. Louis, MO, USA). G418-resistant cell clones were established approximately after 4 weeks.

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from transfected cells or non-transfected cells using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. For the measurement of expression level of miR-424 in the cells, total RNA was reversely transcribed into cDNA with TaqMan MicroRNA Reverse Transcription Kit and the following Real-time PCR was performed using TaqMan Universal Master Mix II (both purchased from Applied Biosystems, Foster City, CA, USA) on the basis of manufacturer's instructions. U6 (Applied Biosystems, Foster City, CA, USA) was used as internal control and the fold changes were calculated by relative quantification $2^{-\Delta\Delta Ct}$ method.

Dual Luciferase Activity Assay

The segment from the 3′-UTR of the MKP-1, which contained the predicted miR-424-binding site, was amplified by PCR and cloned into the pmirGLO vector (Promega, Madison, WI, USA), which referred as MKP-1-wild-type (WT). To form the MKP-1-mutated-type (MKP-1-MT), mutants at the putative binding site of MKP-1-WT were constructed. Then, cells were co-transfected with the reporter construct, control vector, and miR-424 or scramble using Lipofectamine 3000 (Life Technologies, Carlsbad, CA, USA). Luciferase activity was detected by using the dual-luciferase assay system (Promega, Madison, WI, USA) following to the manufacturer's instructions.

Western Blot

The proteins used for Western blotting were extracted from cells using RIA lysis buffer (Beyotime Biotechnology, Shanghai, China) and supplemented with protease inhibitors (Roche, Guangzhou, China). The BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA) was used to quantify the concentration of the proteins. Equivalent proteins (30 μg) were separated using Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). GAPDH antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies were prepared in 5% blocking buffer (BSA) at a dilution of 1:1000. The membranes were incubated with primary antibodies overnight at 4°C, followed by the incubation with secondary antibody marked with horseradish peroxidase (HRP) for 1 h at room temperature. Afterwards, the polyvinylidene difluoride (PVDF) membranes carrying antibodies and blots were transferred onto Bio-Rad ChemiDoc™ XRS system (Bio-Rad, Hercules, CA, USA), and then 200 μL Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) were added to cover the membrane surface. The signals were captured and the intensity of the bands was analyzed using Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All experiments were repeated three times. The results of multiple experiments are presented as mean ± standard deviation. Statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). *p*-values were calculated using a one-way analysis of variance. *p*-value of < 0.05 was considered to indicate a statistically significant result.

Results

OGD Induced Injury in PC-12 Cells

Following OGD of PC-12 cells, cell viability was assessed by CCK-8 assay. It was found that OGD led to significant decrease in the percentage of viable cells ($p < 0.05$; Figure 1A) compared to the control group of PC-12 cells. Next, we estimated the percentage of apoptotic cells by flow cytometry. It was found that the percentage of apoptotic cells was significantly increased following OGD ($p < 0.001$; Figure 1B) compared to the control group. Western blot analysis of the proteins associated with apoptosis revealed

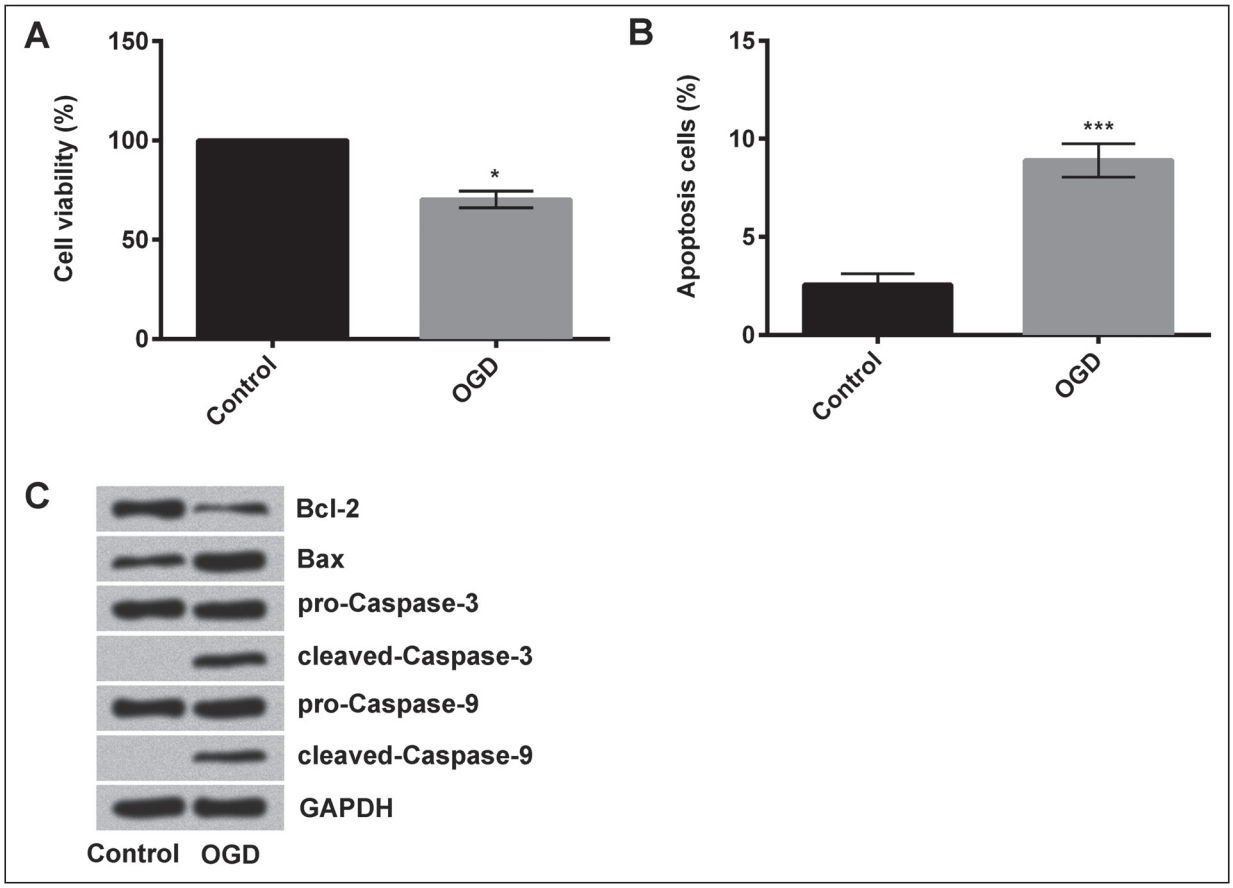

Figure 1. OGD induces the cell injury in PC-12 cells. ***(A)*** Cell viability of PC-12 cells was assessed by CCK-8 assay and ***(B)*** the percentage of apoptotic cells was assessed by flow cytometry. ***(C)*** Western blot analysis was used to measure the expression of apoptosis-related proteins, namely Bax, Bcl-2, cleaved-caspase-3, and cleaved-caspase-9. OGD: oxygen glucose deprivation; CCK-8: cell-counting kit-8. *, $p < 0.05$; ***, $p < 0.001$.

that OGD suppressed expression of anti-apoptotic factor (Bcl-2) and promoted expression of pro-apoptotic factors (Bax, pro-caspase-3, and pro-caspase-9) compared to the control group (Figure 1C). These findings indicate that OGD leads to PC-12 cell damages by suppressing cell proliferation and inducing apoptosis.

Overexpression of miR-424 Reduces OGD-Induced Cell Injury, and Suppression of miR-424 Promotes Cell Injury

qRT PCR analysis revealed that the expression of miR-424 was significantly increased ($p < 0.05$; Figure 2) in PC-12 cells following OGD compared to the control group. Then, PC-12 cells were transfected with either miR-424 mimic or miR-424 inhibitors to alter the expression of miR-424. qRT PCR analysis revealed that the expression of miR-424 was significantly increased ($p < 0.001$; Figure 3A)

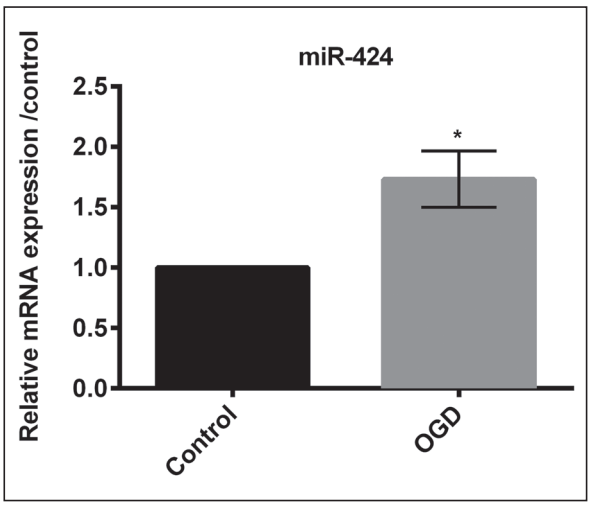

Figure 2. OGD upregulates the expression of miR-424 in PC-12 cells. qRT-PCR was done to estimate the relative mRNA expression of miR-424 in PC-12 cells following OGD. qRT-PCR: quantitative Real-time polymerase chain reaction; OGD: oxygen glucose deprivation.*, $p < 0.05$.

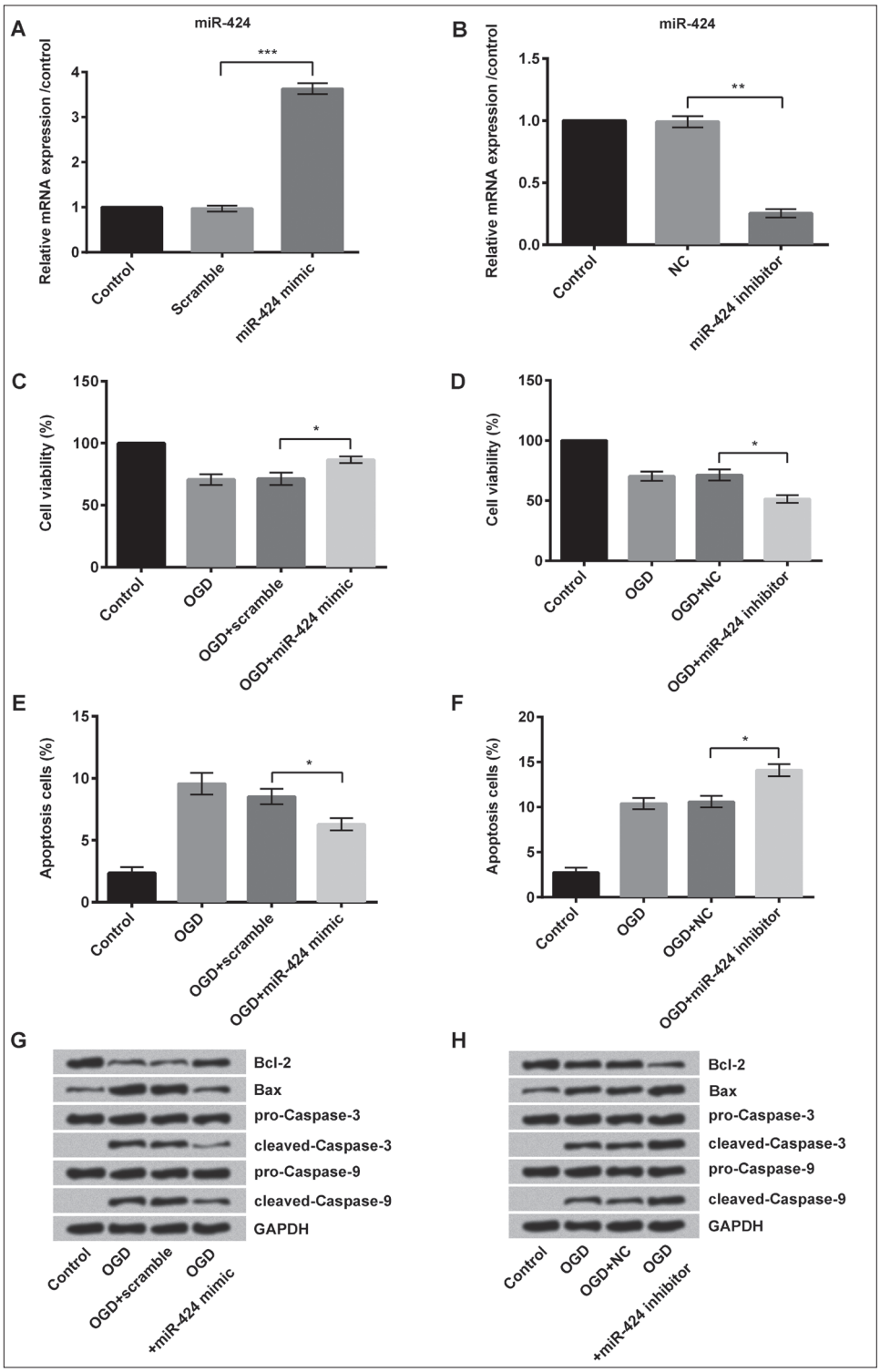

Figure 3. Overexpression of miR-424 reduces OGD-induced cell injury, and suppression of miR-424 promotes cell injury. ***(A*** and ***B)*** PC-12 cells were transfected with scramble (*positive control*), miR-424 mimic, miR-424 inhibitor, and NC (*negative control*). qRT-PCR was done to estimate the relative mRNA expression of miR-424 in the above mentioned groups of cells. ***(C*** and ***D)*** cell viability and ***(E*** and ***F)*** apoptosis were also estimated in these groups of cells by CCK-8 assay and flow cytometry, respectively. ***(G*** and ***H)*** Western blot analysis was used to measure the expression of apoptosis-related proteins, namely Bax, Bcl-2, cleaved-caspase-3, and cleaved-caspase-9 in the above mentioned groups of cells. qRT-PCR: quantitative Real-time polymerase chain reaction; CCK-8: cell counting kit-8; OGD: oxygen glucose deprivation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

in miR-424 mimic group of cells, whereas was significantly decreased ($p < 0.01$; Figure 3B) in miR-424 inhibitor group of cells. Next, we assessed cell viability of these groups of cells using CCK-8. It was found that even in presence of OGD, miR-424 overexpression significantly increased ($p < 0.05$; Figure 3C) the percentage of viable cells and miR-424 suppression led to a significant decrease ($p < 0.05$; Figure 3D) in the same compared to the NC group. Flow cytometry revealed that the percentage of apoptotic cells were significantly lower ($p < 0.05$; Figure 3E) in miR-424 mimic group of cells compared to the scramble group, while suppression of miR-424 expression significantly increased the percentage of apoptotic cells ($p < 0.05$; Figure 3F) compared to the NC group. Western blot analysis of apoptosis-associated proteins revealed that overexpression of miR-424 promoted expression of Bcl-2 and suppressed expression of Bax, pro-caspase-3, and pro-caspase-9 (Figure 3G); suppression of miR-424 expression showed just the opposite results (Figure 3H). These findings indicate that overexpression of miR-424 protects the PC-12 cells from OGD-induced injury.

MKP-1 Was a Target of miR-424

qRT PCR analysis revealed that miR-424 mimic significantly down-regulated the expression of MKP-1 ($p < 0.05$; Figure 4A) in PC-12 cells compared to the scramble group, while suppression of miR-424 expression led to a significant increase in the expression of MKP-1 ($p < 0.01$; Figure 4B) compared to the NC group. Similar results were observed in the protein expression by Western blot analysis (Figure 4C).

Next, to investigate whether miR-424 negatively modulated the expression of MKP-1 through directly targeting its 3'UTR, the relative luciferase activity assay was performed. Two different vectors of MNK-1 were constructed, namely MKP-1-wt and MKP-1-mt. Control and miR-424 mimic group of PC-12 cells were transfected with both the vectors (MKP-1-wt and MKP-1-mt). It was found that luciferase activity of MKP-1-wt was significantly decreased ($p < 0.05$; Figure 4D) in miR-424 mimic group of cells compared to the scramble group. However, luciferase activities of MKP-1-mt were similar in scramble and miR-424 groups of cells (Figure 4D). These findings indicate that MKP-1 is a target of miR-424 and miR-424 negatively regulates the expression of MKP-1.

Suppression of miR-424 Promotes Cell Injury by Upregulating the Expression of MKP-1

Next, we examined the effect of MKP-1 on OGD-induced cell injury in PC-12 cells. Firstly, PC-12 cells were transfected the pEX-MKP-1 and sh-MKP-1, or their corresponding negative controls. The efficiency of transfection was verified by using qRT-PCR and Western blot. The qRT-PCR results showed that pEX-MKP-1 significantly increased the expression of MKP-1 ($p < 0.001$; Figure 5A) and sh-MKP-1 significantly decreased the expression of MKP-1 ($p < 0.01$; Figure 5B). Similar results were found in the Western blot analysis (Figure 5C).

Next, cell viability assay revealed that suppression of MKP-1 expression blocked the effect of miR-424 inhibitor in OGD-injured PC-12 cells, as significantly increased the cell viability in OGD-injured cells ($p < 0.05$; Figure 5D). Flow cytometry analysis revealed that apoptotic cell rates were significantly suppressed ($p < 0.05$; Figure 5E) in the OGD+miR-424 inhibitor+sh-MKP1 group compared with the OGD+miR-424 inhibitor+shNC group. Western blot analysis also confirmed these results (Figure 5F). These findings indicate that suppression of miR-424 expression promotes OGD-induced cell injury via up-regulation of MKP-1 expression.

Overexpression of MKP-1 Promoted OGD-Induced Cell Injury

CCK-8 assay revealed that overexpression of MKP-1 led to significant decrease ($p < 0.05$; Figure 6A) in the percentage of viable cells compared to pEX cells following exposure to OGD. In contrast, suppression MKP-1 expression led to significant increase ($p < 0.05$; Figure 6B) in cell viability even after exposure to OGD. Overexpression of MKP-1 significantly promoted ($p < 0.05$; Figure 6C) apoptosis compared to the pEX control group, and suppression of MKP-1 expression significantly decreased ($p < 0.01$; Figure 6D) the percentage of apoptotic cells compared to the shNC group. Western blot analysis for apoptosis also revealed similar findings (Figures 6E and 6F). These findings indicate that MKP-1 promotes OGD-induced cell injury in PC-12 cells.

MKP-1 Inhibited the Expression of HIF-1α by Inactivation of PI3K/AKT/mTOR Pathways

Western blot analysis revealed that MKP-1 overexpression led to suppression of PI3K/AKT/

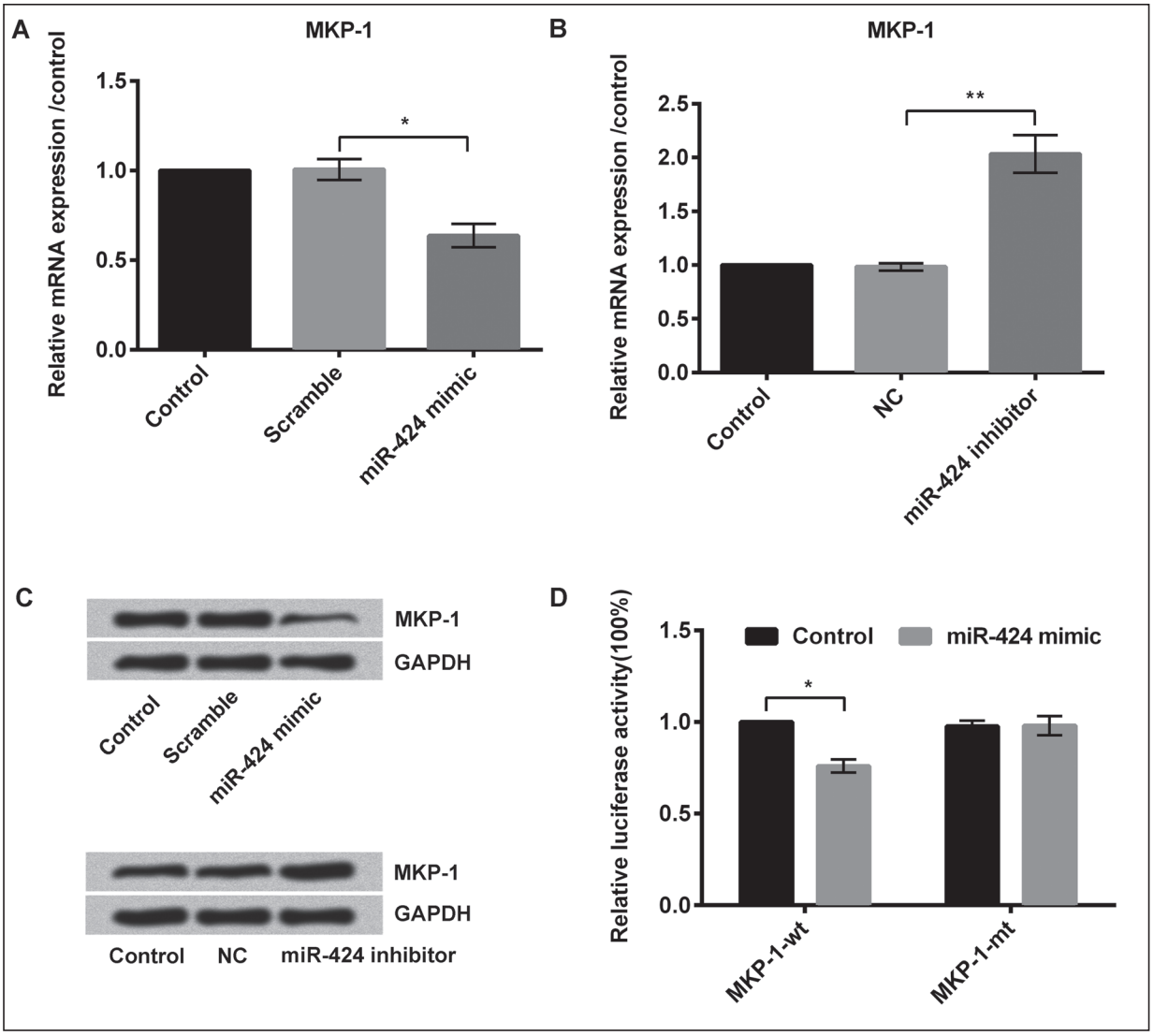

Figure 4. MKP-1 is a target of miR-424. ***(A-C)*** qRT-PCR and Western blot were done to estimate the expressions of MKP-1 in different groups of PC-12 cells. ***(D)*** Two different vectors of MKP-1 were constructed, namely MKP-1-wt (*wild type*) and MKP-1-mt (*mutant type*). Control and miR-424 mimic group of PC-12 cells were transfected with both the vectors (MKP-1-wt and MKP-1-mt). Relative luciferase activity was assessed in the four groups of cells namely control MKP-1-wt, miR-424 mimic MKP-1-wt, control MKP-1-mt, and miR-424 mimic MKP-mt groups of cells. qRT-PCR: quantitative Real- time polymerase chain reaction; MKP-1: mitogen-activated protein (MAP) kinase phosphatase-1; MKP-1-wt: MKP-1 wild type; MKP-1-mt: MKP-1 mutant type; miR-424: microRNA 424. *, $p < 0.05$; **, $p < 0.01$.

mTOR pathways, as suppressed expressions of core factors associated with these pathways, namely p-PI3K, p-AKT, p-mTOR, p-70S6K, and hypoxia-inducible factor-1α (HIF-1α) (Figure 7). Suppression of MKP-1 expression revealed just the opposite results (Figure 7). This finding indicates that MKP-1 promotes OGD-induced cell injury by suppressing the expression of HIF-1α by inactivation of the PI3K/AKT/mTOR pathway.

Discussion

Stroke is one of the leading causes of death worldwide[1-4]. Hence, it remains one of the important research objectives to identify novel drug targets for management of stroke. OGD-induced cell damage has already been used in a number of studies as a model of stroke *in vitro*[17,18]. In the present research, OGD treatment decreased cell

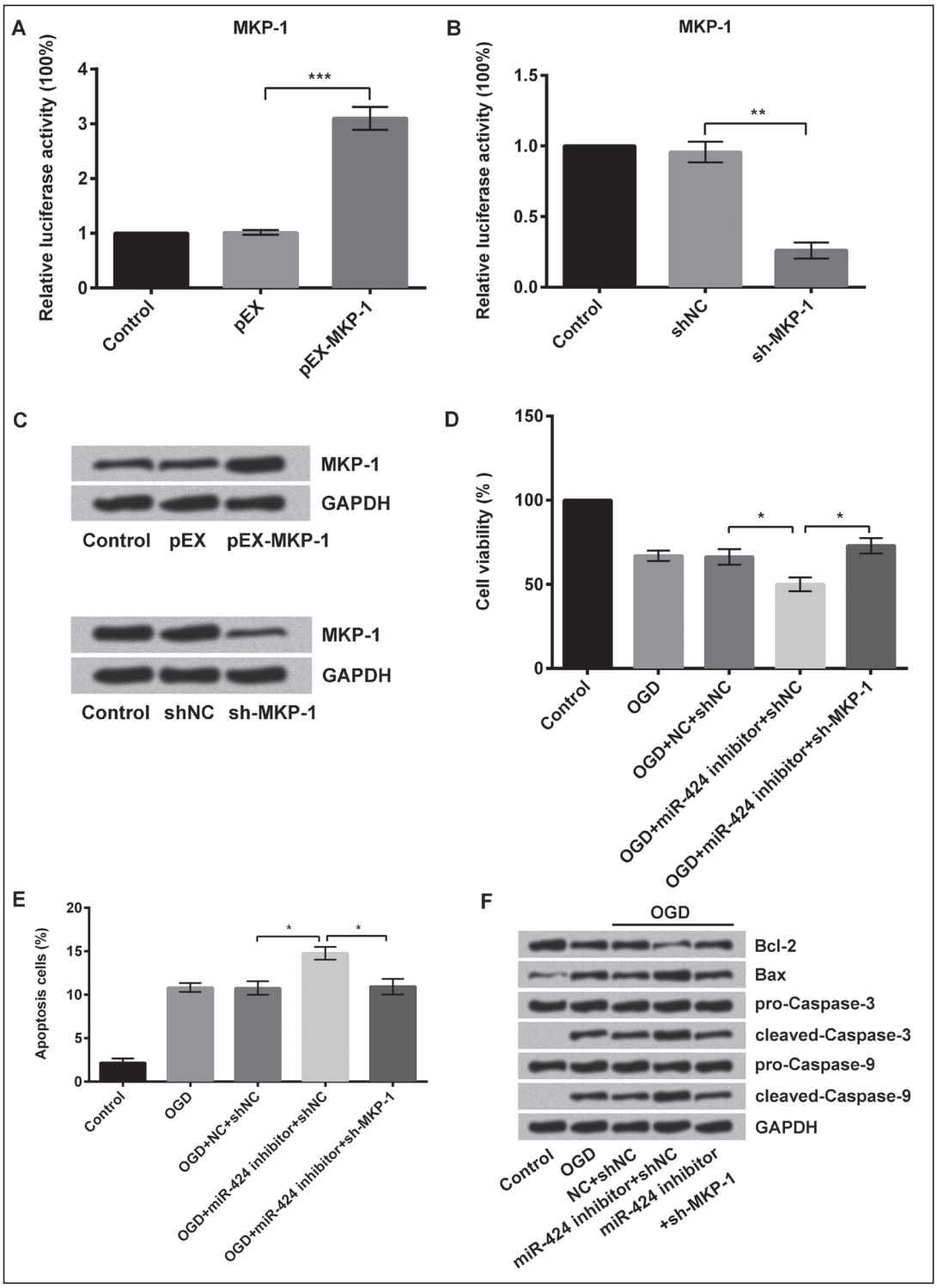

Figure 5. Suppression of miR-424 promotes cell injury by upregulating the expression of MKP-1. ***(A-C)*** pEx-MKP-1 and sh-MKP-1 were transfected in PC-12 cells. qRT-PCR was done to estimate relative mRNA expression of MKP-1 in different group of PC-12 cells namely, control, pEX, pEX-MKP-1, shNC, and sh-MKP-1 following ischemic injury by OGD. ***(D)*** Cell viability and ***(E)*** apoptosis were assessed in different groups of PC-12 cells namely, control, OGD, OGD+NC+shNC, OGD+miR-424 inhibitor+shNC, OGD+miR-424 inhibitor+sh-MKP-1. ***(F)*** Western blot analysis was used to measure the expression of apoptosis-related proteins, namely Bax, Bcl-2, cleaved-caspase-3, and cleaved-caspase-9 in the above mentioned groups of cells. qRT-PCR: quantitative Real-time polymerase chain reaction; MKP-1: mitogen-activated protein (MAP) kinase phosphatase-1; OGD: oxygen glucose deprivation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

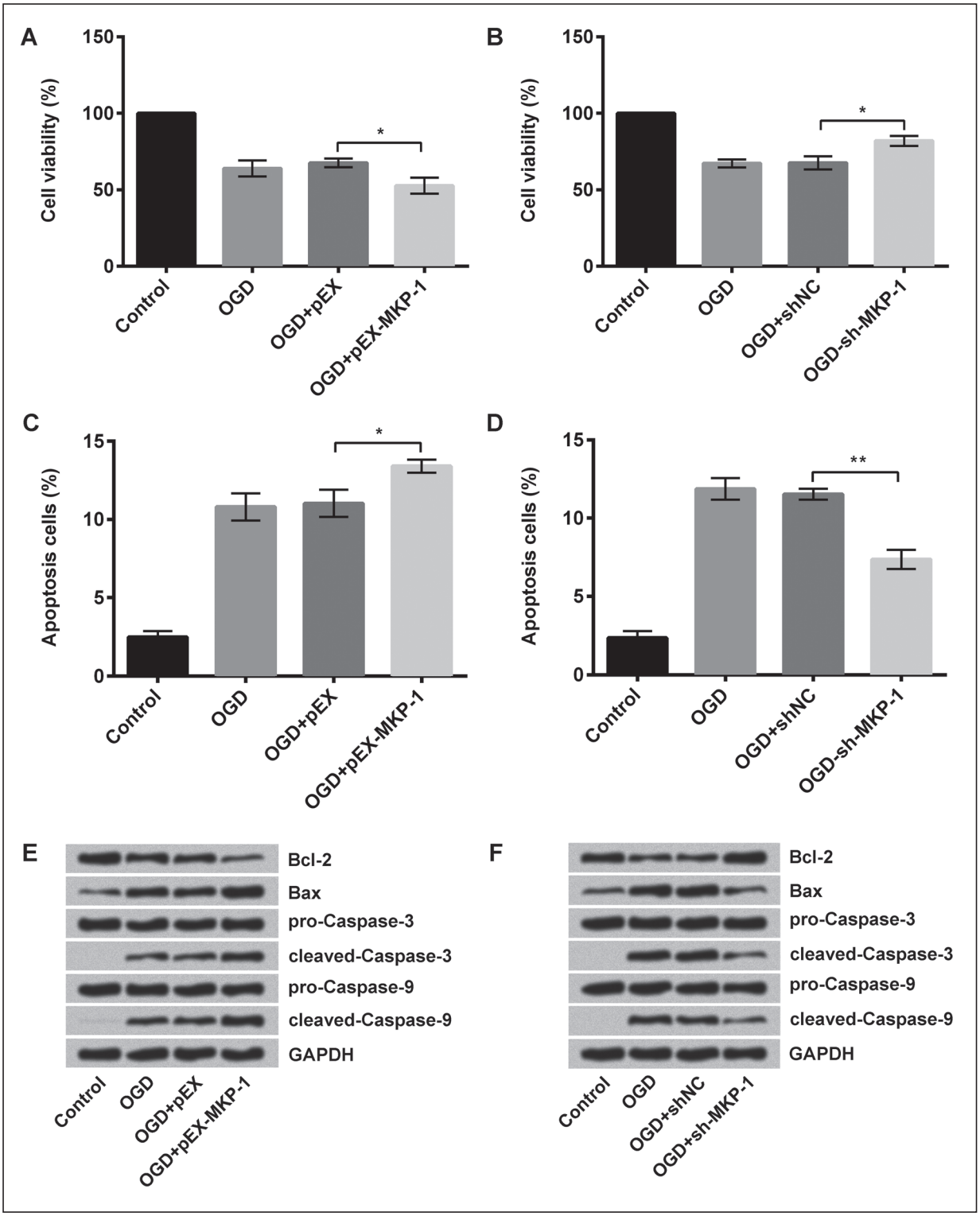

Figure 6. Overexpression of MKP-1 promotes OGD-induced cell injury and suppression of MKP-1 alleviates OGD-induced cell injury. ***(A*** and ***B)*** Cell viability and ***(C*** and ***D)*** apoptosis were assessed using CCK-8 assay and flow cytometry, respectively in different groups of PC-12 cells, namely pEX-MKP-1, sh-MKP-1, pEX, sh-NC following OGD. ***(E*** and ***F)*** Western blot analysis was used to measure the expression of apoptosis-related proteins, namely Bax, Bcl-2, cleaved-caspase-3, and cleaved-caspase-9 in the above mentioned groups of cells. CCK-8: cell counting kit-8; OGD: oxygen glucose deprivation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

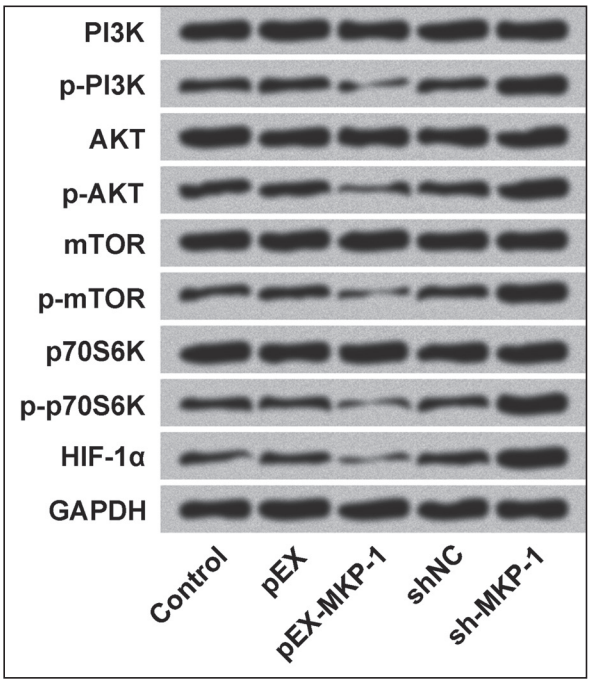

Figure 7. MKP-1 inhibited the expression of HIF-1α by inactivation of PI3K/AKT/mTOR pathways, promoting OGD-induced cell injury. Western blot analysis was done to estimate proteins associated with PI3K/AKT/mTOR pathways, namely p-PI3K, p-AKT, p-mTOR, and p-p70S6K in different groups of cells namely, pEX-MKP-1, sh-MKP-1, pEX, and sh-NC. Western blot also assessed HIF-1α level in these groups of cells. PI3K/AKT/mTOR pathway: phosphoinositide 3-kinase/protein kinase B/mTOR pathway; HIF-1α: hypoxia inducible factor.

viability (Figure 1A) and promoted apoptosis in PC-12 cells (Figures 1B and 1C). Similarly, Fan et al[18] reported that OGD induced acute neuronal injury in PC-12 cells; in this work, prior treatment of PC-12 cells with hydroxysafflor yellow A led to protection of the cells from OGD-induced cell injury. Rajput et al[20] also reported that OGD induced neuronal damage in PC-12 cells.

We found that the expression of miR-424, one of the known miRNAs involved in the pathogenesis of stroke, was induced by OGD (Figure 2). Similar to our findings, two research teams have also established the neuroprotective role of miR-424 in cerebral ischemia[14, 15]. We also found that overexpression of miR-424 suppressed OGD-induced cell injury (Figures 3C, 3E and 3G) and suppression of miR-424 reversed these effects (Figures 3D, 3F and 3H). In line with our findings, Zhao et al[14], reported that the miR-424 acts as its neuroprotective role via suppression of neuronal apoptosis, inhibition of microglial activation, and TNF-α production. Liu et al[15] also reported the neuroprotective role of miR-424 through suppression of oxidative stress. MKP-1 is considered to be the principal phosphatase enzyme responsible for dephosphorylation of MAPKs[21]. Studies[20] have shown that MKP-1 is essential for cell development, growth, differentiation, and maturation. In our study, miR-424 negatively regulated the expression of MKP-1. Furthermore, we found that suppression of MKP-1 alleviated OGD-induced cell injury in PC-12 cells. However, contrary to our findings, Liu et al[22] reported that suppression MKP-1 aggravated cell injury in experimental stroke models. Wancket et al[23] also reported the protective role of MKP-1 in acetaminophen-induced liver damage. HIF-1α is considered to be one of the most important regulators of hypoxia-responsive genes[24,25]. Studies have explored the role of HIF-1α as novel drug target in cerebral ischemia. Shi[25] reported the neuroprotective role of HIF-1α in cerebral ischemia, showing that HIF-1α might exert its neuroprotective role via expression of erythropoietin, vascular endothelial growth factor, and suppression of p53 activation. Liu et al[21] have explored the role of two herbal preparations namely, Catalpol and Peurarin, in cerebral ischemia. They reported that induction of HIF-1α led to induction of PI3K/AKT/mTOR pathway, which collectively facilitated the anti-apoptotic action of Catalpol and Peurarin in cerebral endothelial cells. Similarly, we found that MKP-1-induced suppression of HIF-1α and PI3K/AKT/mTOR pathway facilitated OGD-induced cell damage in PC-12 cells.

Conclusions

Overexpression of miR-424 protects PC-12 cells from OGD-induced cell injury by negatively regulating MKP-1 expression and activation of the PI3K/AKT/mTOR pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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